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INFLUENCE OF TEMPERATURE AND CONCENTRATION ON THE TOXICITY OF SALTS TO FISHES[1]

Edwin B. Powers

Introduction

The study of ecology involves detailed consideration of the physiological relations of animals to their environments. In many fields accuracy has not been attained. One of these is that of the relation of organisms, especially animals, to salts in soils and water. The lack of accurate means of determining the differences in the relations of different species and of different stages in life histories to salts, etc., has led to much confusion. The prevalence of pollution makes it important to know the relations of fishes to toxic amounts of various salts and other chemicals. Other investigators have shown that different species differ, but the differences were usually merely drawn from general experience. It is the purpose of this paper to show that such difference can be determined with precision. It will also be demonstrated that temperature modifies the effect of a given concentration of a substance according to definite laws, and that a rise of one degree at a high temperature has a much greater effect than at a low temperature. In previous work on the toxicities of certain substances to goldfish, the writer found that their physiological activities with few exceptions follow a general law. The present investigation was undertaken to determine the effect of temperature upon this general law and to correlate this effect with the physiology of the organism.

Toxicity and the Measurement of Toxicity

Toxicity has been variously defined by different workers. A common definition is: Any agent which, when introduced into the animal organism, is capable of producing a morbid, noxious, or deadly effect upon it, is said to be toxic. For the purpose of this paper toxicity will be defined as the effect resulting from any agent, the presence of which causes the death of the fish or interferes adversely in any way with its reproduction, development, growth, or normal metabolism. Up to the present time but very little work has been done in determining in a quantitative way the toxicities of naturally occurring and pollution substances. Shelford (1917) has determined the toxicities of a number of the coal tars and coal tar wastes to certain species of fishes by using the one hour survival time as a criterion. Wells has made a quantitative study of the relations of a number of species of fishes to carbon dioxide, lack of oxygen (1913), salts (1915*a*), and carbon monoxide (1918).

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[1] Contributions from the Zoological Laboratory, University of Illinois, No. 154.

In an earlier piece of work experiments were performed to determine the efficiency of the goldfish as a test animal in physiological assay work (Powers, 1918). In this work, it was found that, as the concentration of a substance was increased, beginning with a very dilute solution, a point was reached at which it was first fatal to fish. This was designated as the threshold of lethality concentration.[2] As the concentration was raised from this point the velocity of fatality, which is the reciprocal of the survival time, rose at first very slowly. This was followed by a more rapid rise, and in turn, at higher concentrations, it was less rapid. There is evidence that at still higher concentrations there was again a more rapid rise in the velocity of fatality. In other words, the ratio of increase in toxicity with a given addition in concentration of a solution, as measured by the survival time of the fish, decreases as the concentration is raised. This decrease in the ratio of increase in toxicity for any given addition in the concentration of the substance tested, is more than sufficient to allow for a constant ratio of increase in toxicity for a given percent addition in concentration of the toxic substance. Thus a sigmoid curve is formed when the velocity of fatality is plotted as ordinate, and the concentration of the substance tested is plotted as abscissa. See curve, *CABG,* figure 1. This curve is taken as a representative of the typical form of curve given by all substances tested both in this, and subsequent work, with the exception of cupric chloride, cadmium chloride, and ferric chloride. The deviation of the velocity of fatality curves of these three salts from the normal was explained in a previous work (Powers, 1918). From a study of the curve *CABG*, fig. 1, is evident that at very low concentrations the upward curvature of the velocity of fatality curve is very small as compared to the increment in concentration of the ammonium chloride. At higher concentrations there is a more rapid upward curvature which finally approaches a straight line. From this portion, *i.e.*, the portion *A* to *B,* the upward curvature becomes less, and the velocity of fatality becomes concave downward. Finally, at very high concentrations, there is again a second phase at which the upward curvature is accelerated. This last point is best shown in the velocity of fatality curve (fig. 2) of the straw-colored minnow (*Notropis blennius* Gir.) when killed in ammonium chloride at 19.8° C., because experiments were run over a much wider range of concentrations than the other series. The velocity of fatality curve, fig. 2, approximates a straight line at 0.017 N to 0.03 N., which is followed by a concave downward curvature from 0.03 N. to about 0.09 N., and by a concave upward curvature from about 0.09 N. to 0.6 N.

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[2] The theoretical threshold of lethality concentration is used in preference to theoretical threshold of toxicity concentration of Powers (1918).

This last point has not been demonstrated for all substances tested, but there is evidence that it holds for all showing the first two characteristics.

In all the velocity of fatality curves studied, there is a portion (*A* to *B*, fig. 1) which approaches a straight line, and, in a criterion for the measure of toxicity, can be considered as a straight line. *AB* (fig. 1) when extended as a straight line cuts the *X*-axis at the point *P*. The concentration of ammonium chloride represented on the scale of the graph by the point *P* has been designated as the theoretical threshold of lethality concentration, and the line *PABF* was called the theoretical velocity of fatality curve. Thus, the slope of the theoretical velocity of fatality curve, or the angle θ, fig. 1, can be taken to represent the rapidity with which the rate of velocity of fatality increases with a rise in the concentration of the toxic substance. The theoretical threshold of lethality concentration, and the rate of increase in the velocity of fatality are the reciprocal of each other; *i.e.*, as the theoretical threshold of lethality concentration increases, the toxic activity of the substance can be said to decrease. And when the rate of increase of

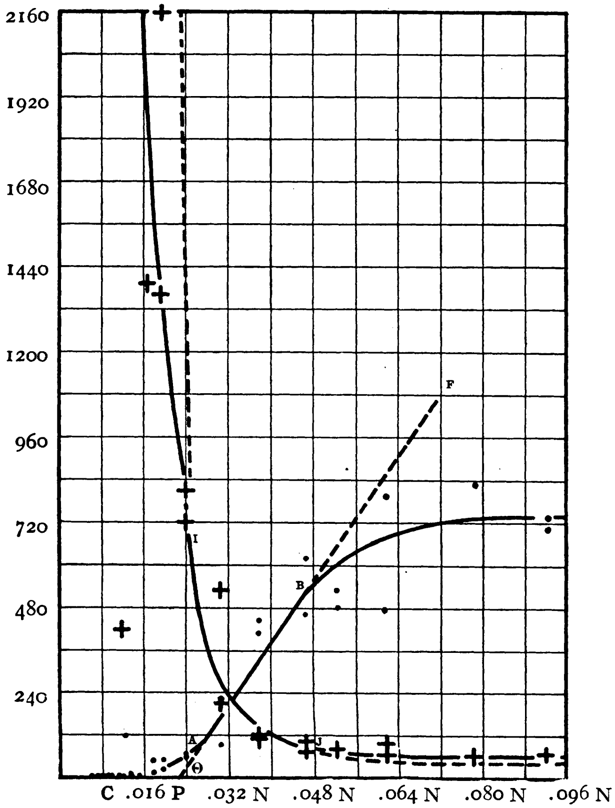

Fig. 1. Relation of concentration of toxic substance to velocity of fatality of goldfish.

velocity of fatality is augmented, the toxic activity can be said to rise. An expression, T (toxicity) $= \sqrt{\frac{\tan\theta}{a}}$, which takes both these factors into consideration has been formulated (Powers, 1918) to express the relative toxicity of a substance. Perhaps a better and more simple expression for the relative toxicity is the equation of the theoretical velocity of fatality curve itself which is the equation of a straight line. That is, $y/(x-a)=K$, where $y=$ the velocity of fatality, $x=$ the concentration of the solution tested, and $a=$ the theoretical threshold of lethality concentration. $k=$ a constant and is taken to represent the relative toxicity of the substance.

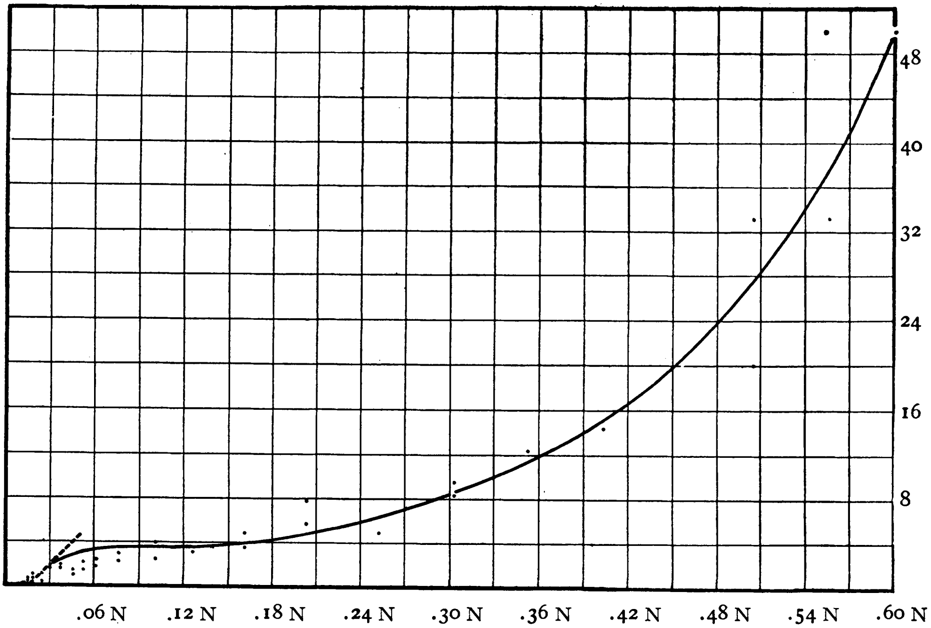

FIG. 2. Velocity of fatality curve.

The equation given above is the equation of a straight line since the theoretical velocity of fatality curve is a straight line (Powers, 1918). The validity of the method is shown by a study of fig. 3. The curves *CABG* and *PABF* represent the velocity of fatality and the theoretical velocity of fatality of the blunt-nosed minnow (*Pimephales notatus* Raf.) when killed in different concentrations of ammonium chloride at 14.2° C., when one block abscissa represents 0.008 N. ammonium chloride; and the curves *C'A'B'G'* and *P'A'B'F'* represent the same data; one block abscissa represents 0.016 N. ammonium chloride. In both sets of curves the ordinate represents velocity of fatality. This is equivalent of having two substances, one of which has just twice the toxic activity of the other, and in which both follow the same law. It can be proved mathematically that k of the equation of the theoretical velocity of fatality curve, *PABF,* and k' of the equation of the theoretical velocity of fatality curve, *P'A'B'F'*, show the same relation; *i.e.*, k equals one half k', and that this relation is not shown by comparing the relative time required to kill the fish in two substances. The same points can be proved when we consider the increased toxic activity of a substance as due to an increased rate of metabolism of the fish, see fig. 4. Figure 4 shows the same velocity of fatality curve, *CABG,* and theoretical velocity of fatality curve *PABF* as in fig. 3, and hypothetical velocity of fatality curve, *CA'B'G'*, and theoretical velocity of fatality curve *PA'B'F'*, in which the effect of the deleterious substance is hypothetically increased by two, due, not to the toxic activity of the substance, but to the increased susceptibility of the fish to the toxic action of the substance as a result of the higher rate of metabolism of the fish. That is, at any concentration of the deleterious substance the velocity of fatality of the fish with the higher rate of metabolism is twice as great as the one with the lower rate of metabolism. This theoretical curve does not hold in its entirety, at least for *Planaria,* as Child (1915 and citations) has shown that at high concentrations of certain toxic substances the *Planaria* with the highest rate of metabolism die first, while at very low concentrations of the toxic substances the order of time until death is reversed. Other factors, such as the size of the individual, the area of sur-

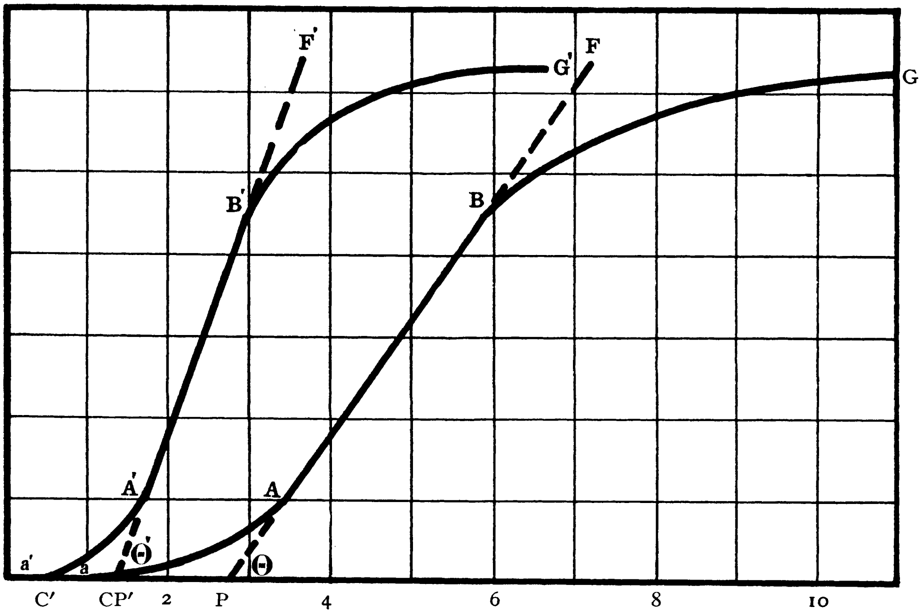

FIG. 3. Velocity of fatality and theoretical velocity of fatality of the blunt-nosed minnow in different concentration of ammonium chloride of 14.2° C.

face, the area of the surface of the gills, and the rates of absorption of the deleterious substances, probably play a part. The hypothetical curve can be taken only as illustrating the tendency of the direction of the velocity of fatality curve, when the increase in the velocity of fatality is due to an increased susceptibility of the fish; and shows that the equation of the theoretical velocity of fatality curve can be taken only as a close approximation of relative toxic values. There is some evidence that the velocity of fatality curves of lithium chloride to goldfish at different temperatures do not all meet at the same point on the X-axis. But this is taken as an approximation of the truth and as a method which can be utilized in obtaining a better understanding of the relation of fishes to deleterious substances.

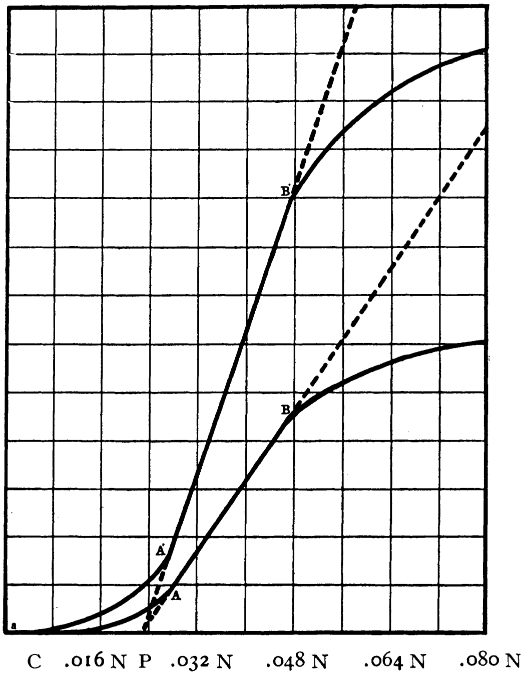

Fig. 4. Same curves as in figure 3, but with effect of the toxic substance increased through higher rate of metabolism of the fish.

Methods and Material

All experiments were performed at controlled temperatures which in no case deviated more than 0.2 to 0.3 of a degree centigrade. These almost uniform temperatures were made possible by the use of a constant temperature bath which consisted of an Alberene stone aquarium 183 cm. by 44.5 cm. by 34.5 cm. deep outside measurements, in which the water stood 15 cm. deep.

The temperature was regulated by the Johnson hot-water thermostat placed at the center of the aquarium. It controlled the flows of hot and cold water which entered the tank at the end opposite the outlet. The temperature of the bath was recorded by a Tycos insertion bulb recording thermometer; it was kept uniform throughout by agitating the water with small widely dispersed bubbles of air.

The fishes to be used in the experiments were kept in running water at a temperature of from 18° to 20° C. The goldfish (*Cerassius cerassius* L.) were obtained of a local dealer and the other fishes were seined from small streams near by. The mortality of the seined fishes was very slight following the second day after they were brought into the laboratory. They were not used in the experiments until the mortality of the stock had ceased, and were rejected after having been kept in the laboratory two or three weeks. In each experiment two fish were placed in 1,500 cc. of a solution contained in a two-quart Mason jar closed with a rubber stopper. The jars were placed in the constant temperature bath and the survival time of each fish recorded.

Distilled water was used in all experiments. This was prepared by condensing through a tube of Jena glass steam from a hot water tank supplied with boiled water. The water when collected was strongly acid from the presence of CO_2, which was removed by aerating with CO_2-free air. After aeration the water gave the following analysis :[1]

Physical examination.				
Turbidity	0			
Color	5			
Odor	2V			
Residue on evaporation.				
Total solids	38	to	42	pts. per million
Alkalinity as calcium carbonte.				
Methyl orange	26	to	36	pts. per million
Chlorides.				
As sodium chloride	0	to	0	pts. per million
Ammonium nitrogen	7.4	to	12.49	pts. per million
Albuminoid ammonia	0.06	to	0.102	pts. per million

This water was not rapidly fatal to fish. Two goldfish lived in it ninety-five and ninety-nine days and six blunt-nosed minnows survived eleven, twelve, thirteen, fifteen, thirty, and thirty-two days respectively. The water was changed every seven days. This specially prepared water was used since it had been found that goldfish would live only three hundred and fifty-two to five hundred and ninety-seven minutes in ordinary distilled water (Powers, 1918).

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[1] Report of Committee on Standard Methods of Water Analysis to the Laboratory of the American Public Health Association, presented at the Havana meeting, Jan. 9, 1905.

EXPLANATION OF EXPERIMENTAL DATA

It has been shown in previous work with goldfish (Powers, 1918) that, when an individual is killed in a toxic substance its survival time is not directly proportional to the concentration of the substance used, but that there is a uniform deviation from this relation which is common to all substances thus far tested, with the exception of the chlorides of some of the heavier metals. Experiments were run with goldfish with lithium chloride, and the blunt-nosed minnow and the straw-colored minnow with ammonium chloride to determine the effect of temperature upon the toxicities of these salts. From these experiments it was hoped to determine the effect of temperature upon rapidity of the toxic action of deleterious substances which might appear in the habitat of the fish either as a natural environmental factor or as a contamination.

In the tables the velocity of fatality which represents the rapidity with which the fish were killed is the reciprocal of the survival time. One hundred, instead of one, over the survival time is used to avoid fractions. The figures are graphic representations of data given in the tables. In all cases the circles (●) and the plus signs (+) represent actual experimental data or calculations made from experimental data.

THE EFFECT OF TEMPERATURE ON THE TOXICITY OF DELETERIOUS SUBSTANCES TO FISHES

(*a*) *The Effect of Temperature on the Toxicity of Lithium Chloride to Goldfish*

A series of experiments was run to determine the effect of temperature on the toxic activity of lithium chloride to goldfish one to two grams in weight. In these experiments the survival time and velocity of fatality of the goldfish was determined over a range of concentrations of lithium chloride from about 0.046 N. to about 0.488 N. between 4° and 34.8° C. at approximately five degree intervals. See table I. By an examination of the data of this table it is seen that, when the survival time or the velocity of fatality of the fish is taken as a criterion for toxicity, the toxic activity of the lithium chloride to the goldfish increases from the lower to the higher temperatures, the greatest increases being at the lowest and highest temperatures. This point will be discussed a little later.

(*b*) *The Effect of Temperature on the Toxicity of Ammonium Chloride to Fishes*

Experiments with ammonium chloride were run with the blunt-nosed minnow at approximately five degree intervals between 14.2° and 24.9° C., and with the straw-colored minnow at 15.6° and 19.8° C. The data on the straw-colored minnow at 19.8° C. is shown graphically in figure 2, lack of space preventing the presentation of this and the other data in tabular form. The velocity of fatality of these fishes was increased with a rise in temperature as was found with the goldfish. The relative increase in the velocity of fatality of the blunt-nosed minnow with rise in temperature will be compared with that of the goldfish a little later.

TABLE I. *Data of goldfish (Carassius carassius L.) when killed in lithium chloride*

Normal	Weight of fish in grams	Survival time of fish in minutes	Velocity of fatality 100/*t*	Temperature centigrade	Normal	Weight of fish in grams	Survival time of fish in minutes	Velocity of fatality 100/*t*	Temperature centigrade
0.058	1.6	1411	0.07	4.0°	0.302	1.2	46	2.17	20.0°
"	2.0	1200	0.08	"	"	1.4	46	2.17	"
0.116	1.5	685	0.15	"	0.418	1.2	30	3.33	"
"	1.6	685	0.15	"	"	1.4	31	3.22	"
0.232	1.0	112	0.89	"	0.488	1.6	35	2.81	"
"	1.7	125	0.80	"	"	1.6	35	2.81	"
0.302	1.2	106	0.94	"	0.058	1.0	322	0.31	24.9°
"	2.2	112	0.89	"	"	2.3	466	0.21	"
0.372	1.2	72	1.37	"	0.116	1.3	152	0.66	"
"	1.2	72	1.37	"	"	1.3	157	0.64	"
0.488	1.2	48	2.08	"	0.232	1.3	37	2.73	"
"	1.9	40	2.50	"	"	1.3	38	2.62	"
0.058	1.8	526	0.19	11.8°	0.302	1.5	27	3.72	"
"	1.8	800	0.12	"	"	2.3	36	2.79	"
0.116	2.8	359	0.28	"	0.372	1.1	23	4.34	"
"	2.8	369	0.27	"	"	2.1	25	4.00	"
0.232	1.7	131	0.76	"	0.046	0.9	224	0.45	29.8°
"	1.7	131	0.76	"	"	1.4	244	0.41	"
0.302	1.2	82	1.22	"	0.058	1.3	224	0.45	"
"	1.9	74	1.35	"	"	1.5	263	0.38	"
0.372	1.9	64	1.59	"	0.093	1.1	98	1.02	"
"	2.1	69	1.45	"	"	1.6	98	1.02	"
0.488	1.6	42	2.35	"	0.116	1.1	110	0.91	"
"	1.9	45	2.22	"	"	1.6	124	0.81	"
0.058	1.5	524	0.19	15.3°	0.151	1.1	44	2.27	"
"	1.5	702	0.14	"	"	1.4	49	2.02	"
0.116	1.7	457	0.22	"	0.232	1.1	25	4.00	"
"	2.3	413	0.24	"	"	2.0	36	2.78	"
0.232	1.2	61	1.64	"	0.267	1.2	19	5.26	"
"	2.2	87	1.15	"	"	1.4	16	6.25	"
0.302	1.0	58	1.72	"	0.302	1.0	22	4.55	"
"	1.0	58	1.72	"	"	2.0	36	2.78	"
0.372	1.1	49	2.04	"	0.337	1.1	14	7.14	"
"	1.2	34	2.91	"	"	1.6	17	5.88	"
0.046	1.0	544	0.18	20.0°	0.372	1.3	17	5.88	"
0.058	1.4	410	0.24	"	"	2.3	18	5.56	"
"	1.5	803	0.12	"	0.488	1.3	10	10.00	"
0.093	1.1	152	0.65	"	0.046	1.4	17	5.88	34.8°
"	2.7	213	0.47	"	"	1.7	50	2.00	"
0.116	1.3	192	0.52	"	0.058	1.3	32	3.63	"
"	1.3	255	0.39	"	"	1.3	41	2.42	"
0.151	1.2	75	1.34	"	0.116	1.2	37	2.73	"
"	1.2	80	1.25	"	"	1.4	61	1.64	"
0.232	0.9	57	1.76	"	0.232	1.1	10	10.00	"
"	1.4	81	1.23	"	"	1.3	15	6.67	"
"	1.0	35	2.86	"	0.302	1.0	8	12.50	"
"	1.0	36	2.78	"	"	1.9	8	12.50	"
0.267	1.4	34	2.94	"	0.372	1.3	7	14.28	"
"	1.4	35	2.86	"	"	1.7	8	12.50	"

(*c*) *The Relation of the Effect of Temperature on the Toxicity of Lithium and Ammonium Chlorides to Fishes and van't Hoff's Rule*

When the data of table I are examined in the light of van't Hoff's rule it is found that, if the actual survival time or the velocity of fatality of the goldfish is taken as a criterion, the ratio of increase in toxicity of the lithium chloride for each approximate rise of ten degrees centigrade gradually increases from the lowest to the highest temperatures tested. Table II gives the ratios for the different concentrations with averages for each approximate ten-degree temperature interval. The temperature interval, 11.8° to 20° C. shows a high average ratio. This is due to individual variation of the fish and to the fact that very exact relative toxicities cannot be deter-

TABLE II. *The ratio of increase in toxicity of lithium chloride to goldfish* (*Carassius carassius L.*) *with an increase of approximately 10 degrees Centigrade*

Normal	4.0° to 11.8° C.	4.0° to 15.3° C.	11.8° to 20 0° C.	15.3° to 24.9° C.	20.0° to 29.8° C.	24.9° to 34.8° C.
0.046	——	——	——	——	2.32	——
0.058	1.97	2.15	1.09	1.56	3.53	10.08
0.093	——	——	——	——	1.87	——
0.116	1.73	1.58	1.60	2.85	1.91	3.15
0.151	——	——	——	——	1.67	——
0.232	0.91	1.60	3.69	1.98	1.16	3.00
0.267	——	——	——	——	1.97	——
0.302	1.35	1.88	3.39	1.86	1.59	3.94
0.337	——	——	——	——	1.97	——
0.372	1.08	1.05	——	1.73	——	3.20
0.488	1.04	——	1.24	——	3.50	——
Averages . . .	1.35	1.65	2.20	2.00	2.15	4.67

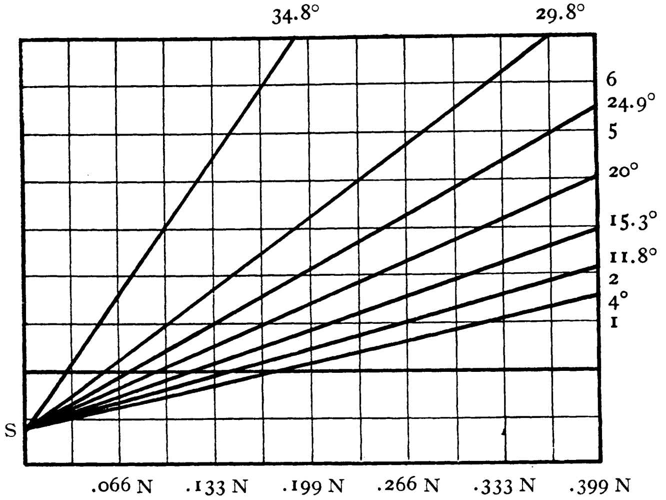

FIG. 5. Theoretical velocity of fatality curves for different temperatures.

mined by comparing the survival time or the velocity of fatality of the fish directly with each other. From this table it is seen that the ratios of the rise of toxic activity of the lithium chloride to the goldfish at lower temperatures is much below and at the higher temperatures is higher than that required by van't Hoff's rule. If the data of table I is plotted in the light of the general relation which the concentration of deleterious substances bears to their toxic activity to fishes, *i.e.*, let the abscissæ represent the concentration of the lithium chloride and the ordinates represent the velocity of fatality of the goldfish and draw the theoretical velocity of fatality curve for each temperature, you obtain the curves shown in figure 5, which is the superposition of the theoretical velocity of fatality curves for each of the temperatures tested. When we determine the relative toxicities of the lithium chloride to goldfish at the different temperatures by means of the equations of the theoretical velocity of fatality curves the individual variations of relative toxicities at different temperatures and the ratio of the increase of toxic activity for a rise of approximately ten degrees centigrade mentioned above disappear to a greater extent. Table III gives the results of these calculations. These results still point to the fact that the ratio of increase in the toxic activity of the lithium chloride for a rise of approximately ten degrees centigrade is much lower at the lower temperatures than that required by van't Hoff's rule. If the data given in column 2 of table III are plotted, a curve can be drawn as shown in *A* of figure 6. This curve has been designated as the temperature toxicity curve. When the Q_{10} is calculated for all the temperatures from 4° to 34° C. on the basis of the toxicity temperature curve (*A* in figure 6) the results given in table IV are obtained. From this table it is plainly seen that the Q_{10} does not meet the demand of van't Hoff's rule, *i.e.*, fall within the limits of 2 to 3, except at the higher temperatures, but that there is a gradual increase of the Q_{10} from the lowest to the highest temperatures, with the greatest increase at the lower and the higher temperatures.

TABLE III. *The relative toxic activities of lithium chloride to the goldfish (Carassius carassius L.) at different temperatures when determined by the equations of the theoretical velocity of fatality curves*

The ratios of increase in the toxicity of the lithium chloride for an approximate rise of ten degrees Centigrade at different temperatures are also given.

Temperature centigrade	Relative toxic activity	Temperatures compared	Ratio of increase in toxity
4°	1.58	4°–15.3°	1.52
11.8°	1.93	11.8°–20°	1.61 –
15.3°	2.40	15.3°–24.9°	1.63 +
20°	3.10	20°–29.8°	1.67 +
24.9°	3.92	24.9°–34.8°	2.55 –
29.8°	5.18		
34.8°	9.99		

The data obtained by killing the blunt-nosed minnow in different concentrations of ammonium chloride at 14.2°, 19.8°, and 24.9° C. have been subjected to the same treatment. When the relative toxic activity of the ammonium chloride to the blunt-nosed minnow at the given temperatures are reduced to relative terms of that of the toxic activity of the lithium chloride to the goldfish at different temperatures it is found that the two sets of data conform closely. See the plus signs (+) figure 6.

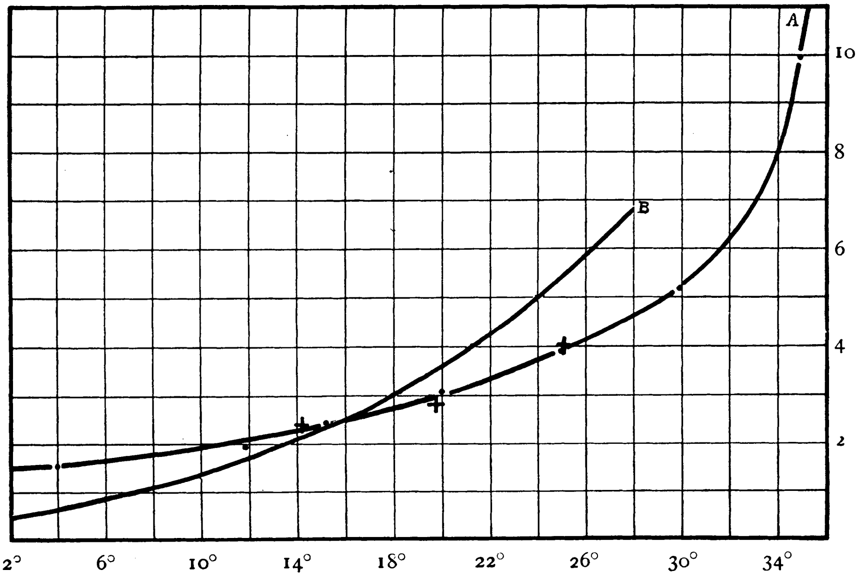

Fig. 6. Temperature toxicity curve (*A*), and Krogh's temperature standard metabolism curve of vertebrates (*B*).

Table IV. *The Q_{10} for the toxicity of lithium chloride to goldfish (Carassius carassius L.).*

The calculations are based upon the temperature toxicity curve to goldfish.

Temperatures centigrade	Q_{10}	Temperatures centigrade	Q_{10}
4° to 14°	1.39	16° to 26°	1.67
6° to 16°	1.47	18° to 28°	1.70
8° to 18°	1.53	20° to 30°	1.75
10° to 20°	1.59	22° to 32°	1.90
12° to 22°	1.64	24° to 34°	2.23
14° to 24°	1.66		

A COMPARISON OF THE TEMPERATURE TOXICITY CURVE WITH THE TEMPERATURE METABOLISM AND THE TEMPERATURE STANDARD METABOLISM CURVES OF OTHER WORKERS

When the data of other workers (see Snyder, 1908 and 1911, for a good bibliography of work done up to that time) are examined it is found that there is a great variation in the effect of rise of temperature or the Q_{10} of physiological and metabolic processes when tested over a very wide range of temperatures. Snyder (1911) says, "in fact, the writer knows of no case where this variation does not occur." Blackman (1905) has said, "As regards the rate of metabolic chemical change in the organism at higher temperatures, this law (van't Hoff's rule) clearly does not express the whole truth." As a general rule there is a decrease in the Q_{10} from the lower to the higher temperatures. Krogh (1916) points out that, "when it is attempted to express the temperature variation of the respiratory exchange by means of the rule of van't Hoff, Q_{10} is found to vary greatly, and in the experiments which have given the most uniform results Q_{10} shows a steady and very large decrease with increasing temperature." In other cases (Loeb and Northrop, 1917, Lillie, 1917, and Groves, 1917) there is no tendency for the Q_{10} to decrease at higher temperatures. In certain of these experiments as well as those of the toxic activity of the lithium chloride to the goldfish there is a sharp upward trend of the Q_{10} at the higher temperatures. This greater increase of the Q_{10} at higher temperatures, at least in the case of the toxic activity of the lithium chloride, is possibly due to an additional factor which has reached its higher points of acceleration. That is, the higher temperatures are nearing the upper limits of temperature endurance of the goldfish. A few of the goldfish died in tap water at 34.8° C. and all died when the temperature was raised above 36° C.

Leitch (1916) in attempting to show that Blackman's (1905) method of extrapolation in calculating the effect of temperature on the growth rate of *Pisum sativum* is untenable has compared his rate of growth curve to Krogh's (1914*b*) standard metabolism curve of vertebrates, and claims that the two are in agreement. But when the temperature toxicity curve of the lithium chloride to goldfish is compared with Krogh's temperature standard metabolism curve, it is found that the two do not coincide (*B* in fig. 6), but that the temperature toxicity curve approximates the square root of the temperature standard metabolism curve. The ratio of the square root of Krogh's temperature standard metabolism curve and the temperature toxicity curve shows a slight rise of the latter above that of the former, both at the lower and higher temperatures. On the other hand the data of Loeb and Northrop (1917, tables IX and X) for the reciprocal of the total duration of life and the duration of life of the imago of the fruit-fly conform fairly well with Krogh's (1914*a, b*) temperature data for the pupæ of *Tenebrio molitor* when relative values of the two sets of data are compared. There are slight variations at the higher temperatures. Lillie's (1917, tables II and III) data conform less exactly with Krogh's temperature metabolism data and show that the velocity of activation of the starfish eggs is higher at the lower and higher temperatures than the relative rate of metabolism of the pupæ of the meal-worm. Sollman, Mendenhall, Stingel (1915) found that the effect of temperature on the time required for systolic standstill of excised frog's heart injected with ouabain corresponded very nearly to the square of the heart's rate quotient. These workers suggested as an explanation, that, on the basis of Weizsaecker's observations, the activity of ouabain is proportional independently, to two factors, viz., the heart's rate and the temperature. That is, when the temperature and the activity of the heart both varied, the activity of the ouabain would increase by the temperature factor multiplied by the rate factor. And they supposed that the temperature affects the rate of the heart and the ouabain response about equally, and thus the temperature would increase the activity by the square of the temperature quotient. The agreement of Loeb and Northrop's data with the temperature metabolism data of the pupæ of the meal-worm is what one would expect, provided the relative effect of temperature on the metabolism of all invertebrates is the same. This last point has not been demonstrated, but Krogh has suggested its probability. Many insects in the imago stage do not take food. Thus you would expect the duration of life, at least in the imago stage of such insects, to be very closely associated with the rate of metabolism. This point is further emphasized by the fact that Krogh (1914) found that the amount of carbon dioxide given off by the pupæ of the meal-worm during the pupal stage is constant for all temperatures at which development is normal. Again the relation of time required to activate the starfish egg with butyric acid (Lillie, 1917) at different temperatures and the temperature metabolism data agree with expectation. That is, according to the hypothesis suggested by Lillie (1915, 1916), the activation process consists essentially in the production of a definite reaction product designated by him as the activating substance. He has shown that the rate at which the butyric acid takes part in the process of activation is directly proportional to its concentration, and suggests that the interaction has the characteristics of a monomolecular reaction. This being true, the temperature should affect the activating and the metabolic processes alike. Thus, the relative increase in the amount of activating substance would be proportional to the relative increase in the rate of metabolism. But the velocity of activation of the starfish eggs is higher than the rate of metabolism of the pupæ of the meal-worm at both the low and high temperatures. This might be explained by the fact that, as Lillie has pointed out, there is a marked activating effect on the eggs at temperatures below 8° and above 28° C. This possibility will account for the higher rates of activation at these temperatures. The fact that the effect of temperature on the toxic activity of lithium chloride to the goldfish follows a curve which is the square root of the temperature standard metabolism curve of vertebrates might be explained on the ground that the lithium chloride attacks some intermediary substance of metabolism. Burge, Neill, and Ashman (1918) have shown that chloroform, ether, magnesium sulphate, and certain other substances attack the catalase of the blood of the cat, dog, and rabbit given these substances. Thus, in this case, one is dealing with the effect of a substance the value of which is equal to the substance introduced. In the case of the starfish eggs, the amount of activating substance that is formed is determined by the rate at which the butyric acid acts on the reacting substance in the cell. Thus the effect of the lithium chloride would have to be considered as that of a reacting substance and not as that of the product of a reaction. Hence, from the law of dynamics, when the two reacting substances are affected at the same rate, the effect on the reaction product would be equivalent to the square of the effect on the reacting substances in a dimolecular reaction, or the effect on the rate of activity of the reacting substances, would be the square root of the effect on the rate of production of the reacting product.

Although there is more or less of an agreement between the square root of Krogh's temperature standard metabolism curve and the temperature toxicity curves of lithium chloride and ammonium chloride, the explanation of the effect of temperature in increasing the activity of a protoplasmic poison is no doubt not so simple. Other factors, such as molecular activity, molecular tension, and metabolic and physiological processes, must be taken into consideration. These suggestions have been made only as a working hypothesis.

The Comparative Resistance of Certain Species of Fishes

The same method that is used to measure the relative toxicity of a substance to fishes can be utilized to determine the relative susceptibility of different species of fishes to a substance. If the relative susceptibility of different species of fishes to any one substance can be taken as the relative susceptibility of the fishes to deleterious substances in general, the relative resistance of the fishes, which is the reciprocal of their susceptibility, can be found. When the equation of the straight line is employed as a criterion for the measurement of the relative susceptibility of fishes to ammonium chloride their relative resistance to this substance is found as follows:

Notropis blennius Gir. (1.5 to 2.0 g.) has a resistance of 1.0
Pimephales notatus Raf. (approximately 2 g.) has a resistance of 1.5
Carassius carassius L. (3.0 to 4.9 g.) has a resistance of 3.0

These calculations are made only for the sizes of fishes mentioned above. The order of the species may not be the same at different stages of the life-history of the fish. This has never been satisfactorily worked out.

General Discussion

It is well known that bodies of water become uninhabitable for some of the most valuable food fishes, both through natural causes and contamination. Certain investigations indicate that the conditions (Shelford, 1911, 1911*a*) and the reactions of the water (Shelford, 1911*b*, and Wells, 1915, 1915*a*) have more to do in determining the habitability of a body of water for food fishes than other factors, such as the availability of food. Shelford (1911*a*) has shown that fish do not always occur where their natural food is most abundant. It has been shown by reaction experiments (Shelford and Allee, 1913, and Wells, 1915, 1915*a*) that fishes generally avoid injurious substances which they encounter in nature. But the avoidance of injurious substances which do not generally occur under natural conditions is not so marked (Shelford, 1917). In fact, fishes may react positively to such substances.

Liebig's law of minimum, as it is generally stated, "The yield of any crop always depends on the nutritive constituent which is present in minimum amount" (Hooker, 1917), can be applied in a modified form to the habitability of a body of water by fishes; *i.e.*, the presence of any one substance may render a body of water uninhabitable for a species of fish. But as Livingston (1917) has stated, "this principle is still quite incomplete logically and its statement will assuredly become more complex as our science advances." The deleterious effect of a substance may depend on the stage of the life-history of the fish, whether it be the developing egg, a young and rapidly growing fry, or an adult. In any of the free moving stages of the life-history, the effect of the injurious substances may be determined by the physiological state of the fish which will cause it to become more sensitive or less sensitive and thus react more definitely or less definitely to any environmental factor. At all stages of the life-history of a fish a deleterious substance may become injurious more rapidly under one set of conditions than under another; or, under still others, it may not be injurious. One of the environmental factors which determine the rapidity with which a substance becomes fatal is temperature, as has been shown by the foregoing experiments. The temperature effect, however, may not be so simple, since the content of the water itself is determined by the season of the year, temperature, light, etc., and the fishes themselves probably have seasonal variations as mentioned above.

All these facts have an important bearing on the problem dealing with the fish in relation to its environment. It is hoped that similar work will be undertaken with other stages of the life-history of the fish, and also that the most sensitive stage will be determined.

Summary of Conclusions

1. The toxicities of the chlorides of lithium and ammonium to fishes are increased with rise of temperature.

2. The effect of temperature on the toxicities of the chlorides of lithium and ammonium does not follow van't Hoff's rule in its entirety.

3. The relative toxic activities of lithium chloride at different temperatures to the goldfish (*Carassius carassius* L.) follow very closely the square root of relative standard metabolism of the vertebrates as given by Krogh.

4. A close approximation of the relative deleterious effect of noxious substances upon fishes can be determined by comparing the constants of the equation of the theoretical velocity of fatality curves of the fishes when killed in these substances. The relative resistance of fishes to these deleterious substances can be determined by the same method since the resistance of the fishes is the reciprocal of the deleterious effect of the substance on the fishes.

5. The relative resistance of *Carassius carassius* L., *Pimephales notatus* Raf., and *Notropis blennius* Gir., to ammonium chloride decreases in the order given.

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